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MEMORANDUM

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Subject: Benchmark dose calculations on thyroid histopathology from the "Effects" Protocol, Argus 1416-003 (Argus, 2001)

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This memo is to transmit results of EPA benchmark dose analysis on thyroid histopathology data in the study entitled *Hormone, Thyroid, and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate during Gestation or via Maternal Milk* (Argus, 2001). The samples used in this analysis were derived from rats treated with ammonium perchlorate (AP) in drinking water during pre-pregnancy, gestation, and lactation at doses of 0.00, 0.01, 0.1, 1.0, and 30 mg/kg/day. Thyroid specimens were removed at necropsy, fixed in 10% formalin, blocked, sectioned, and stained with H & E. For histopathologic evaluation, each tissue was graded separately for colloid depletion, follicular cell hypertrophy, and follicular cell hyperplasia. Data were considered from males and females separately, then combined, as well as the dams, from the following timepoints: gestational day 21 (GD21), post-natal day (PND) 4, PND 9, and PND 21. Because Argus Laboratories identified the day of birth as PND1, the age nomenclature of PND 5, PND 10, and PND22 (Argus, 2001) is off by one day as referenced by EPA definition and these ages are therefore referred to PND 4, PND 9, and PND 21.

Benchmark dose / lower confidence limits on benchmark dose (BMD/BMDL) were generated using the BenchMark Dose Software v. 1.30, publically available from the USEPA. A criterion of a 10% increase in incidence over controls, i.e., BMD10, BMDL10, was adopted for all studies. Data were fit with a log-logistic function constrained such that the slope variable was ≥ 1 :

$$P(\text{response}) = \text{bckgrd} + (1 - \text{bckgrd}) / (1 + e^{-(\text{intercept} + \text{slope} * \log(\text{dose}))})$$

The log-logistic function was chosen as a flexible form given the nature of the observed responses; i.e. non-linear monotonic functions. A chi-squared test was used to determine the goodness-of-fit of these functions. A Weibull function was also fit to these data and yielded nearly identical values. In some cases, the Weibull could not fit the data or needed to have constraints on its exponent relaxed to yield a reasonable fit. For these reasons, we've chosen to tabulate the results of the log-logistic fits (Table 1).

The effects of ammonium perchlorate on the pups' thyroid gland are largely limited to colloid depletion. The dams show additional dose-related effects on thyroid histopathology that were evaluated as thyroid hypertrophy and hyperplasia. The low incidence of these latter two endpoints in pups may be related to the duration of exposure compared to the dams and the adult rats examined in earlier studies (Geller, 2001). Alternatively, hyperplasia and hypertrophy may have been difficult to detect in the smaller thyroid glands from the young pups.

The BMDL10 is lowest in the GD 21 pups (0.12 mg/kg AP for M+F or M alone, 0.04 mg/kg AP for F pups) and increases with age (Figure 1), suggesting that the thyroid gland may be most susceptible to AP effects during gestation or at the time of parturition. This is likely due to the double effects of perchlorate inhibition of thyroid function in the pup, and the lack of protection of the pup by the dam because of her own compromised thyroid function. After 21 days of post-natal exposure the male pups also show follicular cell hyperplasia.

The BMD/BMDL (0.84/0.33) for the PND 4 male + female pups in this study (Table 1) corroborate the BMD/BMDL for colloid depletion for the PND 5 pups from the 1998 Neurobehavioral Developmental study (0.53/0.33) (Geller, 2001a). The BMD/BMDL (17.32/2.17) for the PND 21 male + female pups in this "Effects" study (Table 1) are somewhat higher in this study than in the previous 1999 2-generation reproduction study (2.51/0.80) (Geller, 2001a). However, comparison of the results of the 2-gen study to the current results may be difficult because the differences in the doses tested.

References

Argus Research Laboratories, Inc., 2001. *Hormone, Thyroid, and Neurohistological Effects of Oral (Drinking Water) Exposure to Ammonium Perchlorate in Pregnant and Lactating Rats and in Fetuses and Nursing Pups Exposed to Ammonium Perchlorate during Gestation or via Maternal Milk*. Argus Research Laboratories, Inc., 2001.

Geller, Andrew M. 2001a.. Memo to Annie Jarabek, NCEA, USEPA. Benchmark dose calculations on thyroid histopathology from 1998 data reported after review by Pathology Working Group (Wolf, 2000). 13 November 2001.

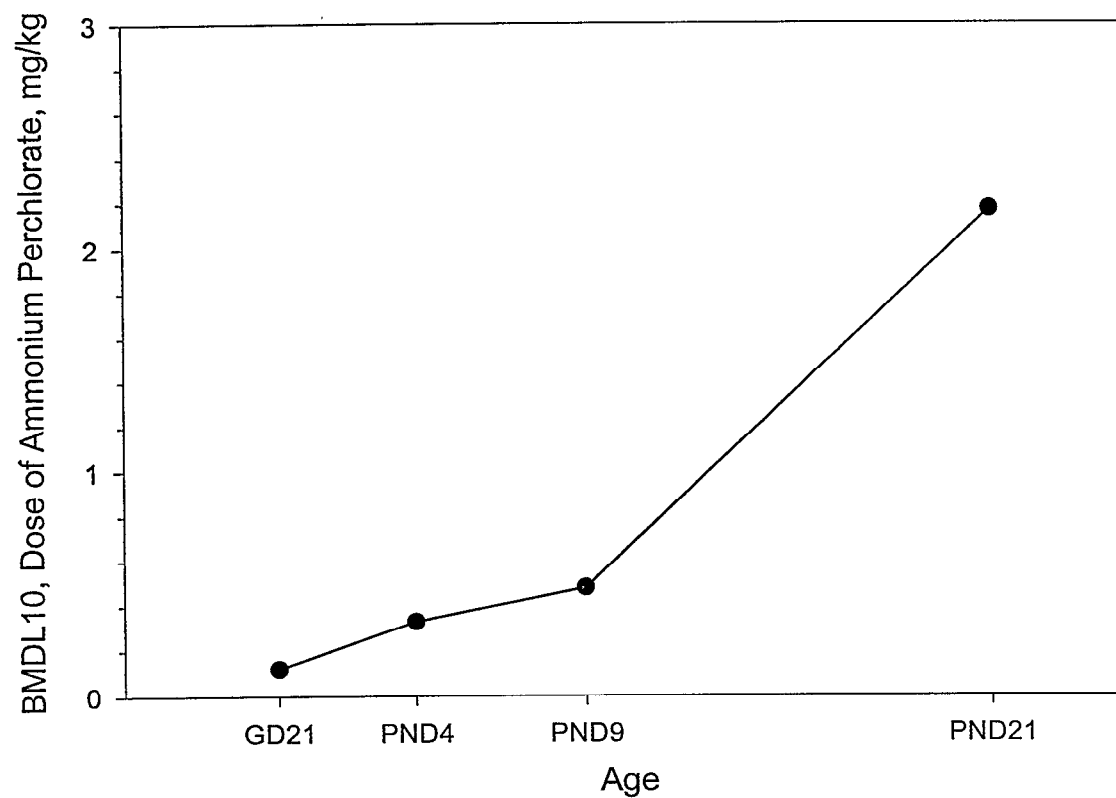


Figure 1. Lower confidence limit on the dose of ammonium perchlorate in drinking water to produce a 10% increase in the incidence of colloid depletion in the thyroid gland as a function of post-natal age of rat pups, male and female data combined.

Table 1: Benchmark dose and benchmark dose lower confidence limits (mg/kg ammonium perchlorate in drinking water) from rat thyroid histopathology data after dosing with ammonium perchlorate. Data is fit with log-logistic function, with exponent restricted to be ≥ 1 . "No effect" signifies no discernable dose-related effect.

Study Population "Effects" Study (Argus, 2001)	BMD BMDL χ^2 p-value slope of logistic function		
	colloid depletion	hypertrophy	hyperplasia
GD 21 Dams	5.10 1.01 1.00 17.90	15.46 1.19 1.00 6.25	28.54 8.51 1.0 5.03
GD 21 Male pups	0.69 0.12 1.00 8.82	No effect	No effect
GD 21 Female pups	0.18 0.04 0.60 2.08	No effect	No effect
GD 21 M + F pups	0.65 0.12 0.16 7.80	No effect	No effect
PND 4 Male pups	0.88 0.29 0.12 7.37	No effect	No effect
PND 4 Female pups	0.82 0.18 0.12 7.78	No effect	No effect
PND 4 M + F pups	0.84 0.33 0.02 7.50	No effect	No effect
PND 9 Dams	0.62 0.13 0.59 2.65	2.65 1.01 0.22 17.86	2.24 0.92 0.49 1.0
PND 9 Male pups	1.29 0.71 0.59 6.40	No effect	No effect
PND 9 Female pups	0.33 0.13 0.61 1.30	No effect	No effect
PND 9 M + F pups	0.93 0.48 0.36 3.77	No effect	No effect
PND 21 Dams	1.21 0.62 0.34 4.90	15.60 1.24 1.0 6.34	3.59 0.99 0.66 1.0
PND 21 Male pups	17.33 1.36 1.0 5.85	No effect	26.97 5.45 0.58 5.06
PND 21 Female pups	16.42 1.24 1.00 5.94	No effect	No effect
PND 21 M + F pups	17.32 2.17 1.0 5.92	No effect	54.17 13.70 0.24 1.0